29 COCAINE, COCAETHYLENE AND BENZOYLECGONINE QUANTITATION AND CONFIRMATION BY LIQUID/LIQUID EXTRACTION AND GCMS

29.1 Summary

29.1.1 Cocaine (COC) and cocaethylene (CE) are extracted from biological samples by making the samples basic with saturated borate buffer and extracting with toluene/hexane/isoamyl alcohol (THIA). The remaining aqueous layer is re-extracted with a more polar organic solvent (chloroform/ethanol) to recover benzoylecgonine (BE). The BE extracts are derivatized with n-propyl iodide in dimethylsulfoxide (DMSO) in the presence of trimethylsulfonium hydroxide (TMSH). Both the COC/CE and BE extracts are analyzed by GCMS for confirmation and quantitation by selected ion monitoring.

29.2 Specimen Requirements

29.2.1 3 mL of whole blood, biological fluids or tissue homogenates.

29.3 Reagents And Standards

29.3.1 Cocaine hydrochloride

29.3.2 Cocaethylene

29.3.3 Benzoylecgonine

29.3.4 Benzoylecgonine-d_3, 100 µg/mL

29.3.5 Methapyrilene

29.3.6 Sodium tetraborate decahydrate

29.3.7 Toluene

29.3.8 Hexane

29.3.9 Isoamyl alcohol

29.3.10 Sodium hydrogen carbonate

29.3.11 Potassium carbonate

29.3.12 Sulfuric Acid

29.3.13 Dimethyl sulfoxide (DMSO)

29.3.14 Acetonitrile

29.3.15 Chloroform (do **NOT** use Burdick-Jackson chloroform with amylene preservative)

29.3.16 Ethanol

29.3.17 Trimethysulfonium iodide

29.3.18 Silver oxide

29.3.19 Methanol

29.3.20 1-Iodopropane

29.3.21 Sodium sulfite

29.4 Solutions, Internal Standards, Calibrators, Controls

29.4.1 Saturated borate buffer solution. Add sodium tetraborate decahydrate to dH_2O until no more dissolves after shaking vigorously.

29.4.2 Toluene:Hexane:Isoamyl Alcohol (THIA) extraction solvent (78:20:2), v:v:v: Mix toluene (780 mL), hexane (200 mL), and isoamyl alcohol (20 mL).

29.4.3 Sodium Hydrogen Carbonate/Potassium Carbonate (dry 3:2 w/w) Mix 300 g $NaHCO_3$ with 200 g K_2CO_3

29.4.4 0.5 N Sulfuric Acid. Pipet 7 mL concentrated sulfuric acid into a 500 mL volumetric flask and QS to volume with dH_2O.

29.4.5 Chloroform/ethanol (4:1, v/v): Mix 400 mL chloroform with 100 mL ethanol.

29.4.6 Trimethyl sulfonium hydroxide (TMSH): Add 1.38 g trimethyl sulfonium iodide and 1.8 g silver oxide to 5 mL methanol in teflon capped foil covered tube (reacts to light). Rotate 4 hours or more. Centrifuge at approximately 2000 rpm for 10 minutes. Decant supernatant into clean Teflon capped foil covered tube. Store in freezer.

29.4.7 Working stock solutions

29.4.7.1 Cocaine stock solution (1 mg/mL). Weigh 11.2 mg cocaine hydrochloride into a 10 mL volumetric flask and QS to volume with acetonitrile.

29.4.7.2 Benzoylecgonine stock solution (1 mg/mL). Weigh 10 mg benzoylecgonine into a 10 mL volumetric flask and QS to volume with methanol.

29.4.7.3 Cocaethylene stock solution (1 mg/mL). Weigh 10 mg cocaethylene into a 10 mL volumetric flask and QS to volume with acetonitrile.

29.4.7.4 COC/CE/BE working stock solution (0.1 mg/mL). Pipet 0.5 mL each of 1 mg/mL cocaine, cocaethylene and benzoylecgonine stock solution into a 5 mL volumetric flask and QS to volume with methanol. Prepare fresh daily.

29.4.8 Internal Standard Solutions

29.4.8.1 Methapyrilene stock solution (1 mg/mL). Weigh 28.5 mg methapyrilene into a 25 mL volumetric flask and QS to volume with methanol.

29.4.8.2 Methapyrilene working stock solution (0.1 mg/mL, internal standard for COC and CE). Pipet 10 mL of 1 mg/mL methapyrilene stock solution into a 100 mL volumetric flask and QS to volume with methanol. Solution may be stored refrigerated for one year.

29.4.8.3 BE-d_3 working solution (0.1 mg/mL, internal standard for BE). Pipet 1 mL of 1 mg/mL BE-d_3 into a 10 mL volumetric flask and QS to volume with methanol.

29.4.9 The following is an example of an acceptable procedure for the preparation of calibrators. Other quantitative dilutions may be acceptable to achieve similar results. To appropriately labeled 16 x 125 mm screw cap tubes, add the following volumes of the 0.1 mg/mL COC/CE/BE working solution and 3 mL blank blood to obtain the final calibrator concentrations:

29.4.9.1 Cal 1: 0.03 mg/L: 1 µL of 0.1 mg/mL working standard + 3 mL blank blood

29.4.9.2 Cal 2: 0.05 mg/L: 1.5 µL of 0.1 mg/mL working standard + 3 mL blank blood

29.4.9.3 Cal 3: 0.10 mg/L: 3 µL of 0.1 mg/mL working standard + 3 mL blank blood

29.4.9.4 Cal 4: 0.25 mg/L: 7.5 µL of 0.1 mg/mL working standard + 3 mL blank blood

29.4.9.5 Cal 5: 0.50 mg/L: 15 µL of 0.1 mg/mL working standard + 3 mL blank blood

29.4.9.6 Cal 6: 1.0 mg/L: 30 µL of 0.1 mg/mL working standard + 3 mL blank blood

29.4.9.7 Cal 7: 2.0 mg/L: 60 µL of 0.1 mg/mL working standard + 3 mL blank blood

29.4.9.8 Cal 8: 4.0 mg/L: 120 µL of 0.1 mg/mL working standard + 3 mL blank blood

29.4.10 Controls

29.4.10.1 Negative blood control. Blood bank blood (or comparable) determined not to contain cocaine, cocaethylene or benzoylecgonine.

29.4.10.2 QAS Toxicology Control: 0.1 mg/L cocaine and cocaethylene and 1.0 mg/L benzoylecgonine

29.4.10.3 In house control is prepared from a different lot number or a different manufacturer of cocaine, cocaethylene and benzoylecgonine.

29.5 Apparatus

29.5.1 Agilent GC/MSD, Chemstation software, compatible computer & printer

29.5.2 Test tubes, 16 x 125 mm round bottom, screw cap tubes, borosilicate glass with Teflon caps

29.5.3 Test tubes, 16 x 114 mm (10 mL) glass tubes, conical bottom

29.5.4 Centrifuge capable of 2000 – 3000 rpm

29.5.5 Vortex mixer

29.5.6 Heating block

29.5.7 Test tube rotator

29.5.8 Evaporator/concentrator

29.5.9 GC autosampler vials and inserts

29.5.10 GC/MSD parameters. Instrument conditions may be changed to permit improved performance.

29.5.10.1 Acquisition Mode: SIM

29.5.10.2 SIM ions:

29.5.10.2.1 COC: 303, 272, 182

29.5.10.2.2 CE: 317, 272, 196

29.5.10.2.3 BE: 210, 272, 331

29.5.10.2.4 BE-d_3: 213, 275, 334

29.5.10.2.5 Methapyrilene 261, 191, 97

29.5.10.3 Column: HP 5MS 25 m x 0.25 mm x 0.25 µm

29.5.10.4 Detector Temperature: 280° C

29.5.10.5 Oven Program for COC/CE

- Equilibration time: 0.50 minutes
- Initial temp: 125° C
- Initial time: 1.5 minutes
- Ramp: 20° C/min
- Final Temp: 280° C
- Final Time: 3.5 minutes

29.5.10.6 Oven Program for BE

- Equilibration time: 0.50 minutes
- Initial temp: 125° C
- Initial time: 2 minutes
- Ramp1: 35° C/min
- Final Temp 1 : 180° C
- Ramp 2: 40° C/min
- Final Temp 2: 280° C
- Final Time: 2 minutes

29.5.10.7 Inlet

- Mode: Splitless
- Temperature: 250° C
- Injection volume: 2.0 µL
- Purge Time: ON at 2.0 minute

29.6 Procedure

29.6.1 Label clean 16 x 125 mm screw cap tubes accordingly, negative, calibrators, control(s) and case sample IDs.

29.6.2 Pipet 3 mL of blank blood, calibrators, controls and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes.

29.6.3 Add 10 µL of 0.1 mg/mL methapyrilene internal standard to all tubes and vortex briefly.

29.6.4 Add 50 µL of 0.1 mg/mL BE-d3 internal standard to all tubes and vortex briefly.

29.6.5 Add 3 mL saturated borate buffer to each tube.

29.6.6 Add 6 mL THIA (78:20:2) extraction solvent to each tube.

29.6.7 Cap and rotate tubes for 20 minutes.

29.6.8 Centrifuge at approximately 2000 rpm for 15 minutes.

29.6.9 Transfer upper (organic) layer to appropriately labeled 13 x 100 mm screw cap tubes and set aside for COC/CE extraction.

29.6.10 To the lower aqueous layers (BE), add 7 mL chloroform/ethanol extraction solvent. Add solvent to no more than 4 tubes at a time. Cap immediately and invert tubes for 1 minute to prevent the formation of large clumps/clots.

29.6.11 Rotate tubes for 10 minutes.

29.6.12 Centrifuge at approximately 2000 rpm for 15 minutes.

29.6.13 Aspirate and discard upper (aqueous) layer. Break protein plug. Carefully remove lower organic layer (filter if necessary) and transfer to appropriately labeled 13 x 100 mm screw cap tubes.

29.6.14 Evaporate samples to dryness at 60-65° C under nitrogen.

29.6.15 Add 500 µL DMSO, 100 µL 1-iodopropane and 100 µL TMSH to each tube.

29.6.16 Cap each tube, swirl gently and heat at 60-65° C for 5 minutes.

29.6.17 Remove tubes from heat, swirl gently and let stand 45-60 minutes for cooling.

29.6.18 Add 0.2 mL of 0.5 N H_2SO_4 to each tube and swirl gently.

29.6.19 Add several mg sodium sulfite to each until samples turn white.

29.6.20 Add 2 mL THIA extraction solvent to each tube.

29.6.21 Cap tubes and invert approximately 20-30 times.

29.6.22 Centrifuge at approximately 2000 rpm for 10 minutes.

29.6.23 Aspirate and discard upper (organic) layer.

29.6.24 Adjust remaining aqueous layer to a basic pH by slowly adding solid 3:2 $NaHCO_3/K_2CO_3$ buffer until effervescence ceases. Then add approximately 0.3 gm excess $NaHCO_3/K_2CO_3$ buffer to saturate the aqueous layer.

29.6.25 Add 400 µL THIA extraction solvent to each tube. Cap and vortex tubes for 10-15 seconds.

29.6.26 Centrifuge at approximately 2000 rpm for 5 minutes.

29.6.27 Carefully transfer (upper) organic layer to appropriately labeled GC autosampler vials for BE analysis.

29.6.28 To organic layers from 29.6.9, begin isolating COC/CE by adding 2 mL 0.5 N sulfuric acid.

29.6.29 Cap and rotate tubes for 20 minutes.

29.6.30 Centrifuge at approximately 2000 rpm for 15 minutes.

29.6.31 Aspirate and discard top (organic) layer.

29.6.32 Adjust remaining aqueous layer to a basic pH by slowly adding solid 3:2 $NaHCO_3/K_2CO_3$ buffer until effervescence ceases. Then add approximately 0.3 gm excess $NaHCO_3/K_2CO_3$ buffer to saturate the aqueous layer.

29.6.33 Add 400 µL THIA extraction solvent to each tube. Cap and vortex tubes for 10-15 seconds.

29.6.34 Centrifuge at approximately 2000 rpm for 5 minutes.

29.6.35 Carefully transfer upper (organic) layer to appropriately labeled GC autosampler vials for COC/CE analysis.

29.7 Calculations

29.7.1 Calculate the concentrations by interpolation of a linear plot of the response curve based on peak height (or area) ratios (using the target ions listed under GCMS conditions) versus calibrator concentration.

29.8 Quality Control

29.8.1 See Toxicology Quality Guidelines

29.9 References

29.9.1 J Valentour, V Aggarwal, M McGee and S Goza. Cocaine and benzoylecgonine determinations in postmortem samples by GC. J Anal Tox 2: 134-137, 1978.

29.9.2 V Spiehler and D Reed. Brain concentrations of cocaine and benzoylecgonine in fetal cases. J For Sci 30: 1003-1011, 1985